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ab233497
Histone H3 (tri-methyl K4) Quantification Kit (Colorimetric, Circulating)

For detecting circulating Histone H3 (tri-methyl K4) in plasma and serum from human, mouse or rat.

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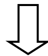
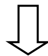
1. Overview

Histone H3 (tri-methyl K4) Quantification Kit (Colorimetric, Circulating) (ab233497) is designed for measuring total H3K4me3 in plasma or serum. In an assay with this kit, the Histone H3 proteins trimethylated at K4 in the plasma/serum sample are captured on the strip wells coated with anti-H3K4me3 antibody. The captured H3K4me3 proteins can be then recognized with detection antibody followed by a color development reagent. The ratio of H3K4me3 is proportional to the intensity of absorbance. The absolute amount of H3K4me3 can be quantitated by comparing to the standard control.

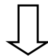
Bind Histone H3 (tri-methyl K4) to assay wells.



Wash wells, then add detection antibody solution.



Wash and add color developer solution.



Measure absorbance at Ex/Em = 450/655 nm.

2. Materials Supplied and Storage

Store Detection Antibody and Standard Control at –20°C away from light. Store Wash Buffer, Histone Assau Buffer, Developer Solution, 8 Well Assay Strips and Control Assay Strips at 4°C away from light. Store Stop Solution and Adhesive Covering Film at room temperature away from light. Kit can be stored for 6 months from receipt, if components have not been reconstituted.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	48 tests Quantity	96 tests Quantity	Storage temperatur e (before prep)
WB (10X Wash Buffer)	14 mL	28 mL	4°C
HAB (Histone Assay Buffer)	4 mL	8 mL	4°C
DS (Developer Solution)	5 mL	10 mL	4°C
SS (Stop Solution)	5 mL	10 mL	RT
8-Well Assay Strips (With Frame)	4	10	4°C
Control Assay Strips (With Frame)#	2	2	4°C
Adhesive Covering Film	1	1	RT
DAb (Detection Antibody, 1000X)*	6 µL	12 µL	-20°C
Standard Control (100 µg/ml)	10 µL	20 µL	-20°C

*Spin the solution down to the bottom prior to use.

#Control Assay Strips are green trimmed for distinguishing from 8 Well Assay Strips (for samples). The control Assay Strips are only for control use and should not be used for sample assay.

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Adjustable pipette or multiple-channel pipette.
- Multiple-channel pipette reservoirs.
- Aerosol resistant pipette tips.
- Microplate reader capable of reading absorbance at 450 nm.
- 1.5 ml microcentrifuge tubes.
- Incubator for 37°C incubation.
- Distilled water.
- Plasma or serum.
- Parafilm M or aluminium foil.

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 WB (10X Wash Buffer)

5.1.1 For a 48-reaction size kit, prepare diluted 1X Wash Buffer by adding 13 mL of 10X Wash Buffer to 117 mL of distilled water and adjust pH to 7.2-7.5.

5.1.2 For the 96-reaction size kit, add 26 mL of 10X Wash Buffer to 234 mL of distilled water and adjust pH to 7.2-7.5.
Δ Note Diluted 1X Wash Buffer can now be stored at 4°C for up to six months.

5.2 HAB (Histone Assay Buffer)

Ready to use as supplied.

5.3 DS (Developer Solution)

Ready to use as supplied.

5.4 SS (Stop Solution)

Ready to use as supplied.

5.5 8-Well Assay Strips (With Frame)

Ready to use as supplied.

5.6 Control Assay Strips (With Frame)

Ready to use as supplied.

5.7 Adhesive Covering Film

Ready to use as supplied.

5.8 DAb (Detection Antibody, 1000X)

Dilute DAb with Diluted WB 1X Wash Buffer at a ratio of 1:1000 (i.e., add 1 µl of DAb to 1000 µl of Diluted WB). 50 µl of Diluted DAb will be required for each assay well.

5.9 Standard Control (100 µg/ml)

Ready to use as supplied.

Δ Note Keep each of the individual solutions (except diluted 1X Wash Buffer) on ice until use. Any remaining diluted solutions, other than the diluted 1X Wash Buffer, should be discarded if not used within the same day.

6. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

First, dilute Standard Control to 50 ng/µl by adding 5 µl of Standard Control to 5 µl of HAB (Histone Assay Buffer) and to 5 ng/µl by adding 1 µl of Standard Control to 19 µl of HAB. Then, further prepare seven concentrations by using the 5 ng/µl and 50 ng/µl of Diluted Standard Control with HAB into final concentrations of 0.5, 1, 2, 5, 10, 20, and 50 ng according to the following dilution chart:

Tube	Standard Control (5 ng/µL)	Standard Control (50 ng/µL)	HAB	Resulting Concentration
1	1.0 µL	-	9.0 µL	0.5 ng/µL
2	1.0 µL	-	4.0 µL	1 ng/µL
3	2.0 µL	-	3.0 µL	2 ng/µL
4	4.0 µL	-	-	5 ng/µL
5	-	1.0 µL	4.0 µL	10 ng/µL
6	-	2.0 µL	3.0 µL	20 ng/µL
7	-	4.0 µL	-	50 ng/µL

7. Sample Preparation

General sample information:

The amount of plasma or serum for each assay can be 10 to 40 µl with an optimal amount of 30 µl.

The standard control is provided in this kit for the quantification of circulating histone H3K4 trimethylation. Because content of H3K4me3 can vary from different individuals and from normal and diseased states, it is advised to run replicate samples to ensure that the signal generated is validated.

8. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.
- Review the configuration of the strip-well plate setup for standard curve preparation in a 48-assays format below (for a 96-reaction format, strips 7 through 12 can be configured as Sample). The controls and samples can be measured in duplicate, loaded vertically instead of horizontally.

8.1 Histone Binding:

1. Predetermine the number of strip wells required for your experiment. It is advised to run replicate samples (include blank and positive controls) to ensure that the signal generated is validated. Carefully remove unneeded strip wells from the plate frame and place them back in the bag (seal the bag tightly and store at 4°C).
2. For blank wells: Add 50 µl of HAB to each well.
3. For standard wells: Add 50 µl of HAB and 1 µl of Diluted Standard Control to each standard well, each at a different concentration between 0.5 and 50 ng/µl (based on the dilution chart in section 6; refer to the following table as an example).

Well	Strip 1	Strip 2	Strip 3	Strip 4	Strip 5	Strip 6
A	Blank	Blank	Sample	Sample	Sample	Sample
B	SC 0.5 ng	SC 0.5 ng	Sample	Sample	Sample	Sample
C	SC 1 ng	SC 1 ng	Sample	Sample	Sample	Sample
D	SC 2 ng	SC 2 ng	Sample	Sample	Sample	Sample
E	SC 5 ng	SC 5 ng	Sample	Sample	Sample	Sample
F	SC 10 ng	SC 10 ng	Sample	Sample	Sample	Sample
G	SC 20 ng	SC 20 ng	Sample	Sample	Sample	Sample
H	SC 50 ng	SC 50 ng	Sample	Sample	Sample	Sample

4. For sample wells: Add 50 µL of HAB and 30 µl of your plasma or serum sample.
   Δ Note Follow the suggested well setup diagram in section 8.1.3).
5. Tightly cover strip-well microplate with Adhesive Covering Film to avoid evaporation and incubate at 37°C for 60 min.
   Δ Note The Adhesive Covering Film can be cut to the required size to cover the strips based on the number of strips to be used.
6. Remove the reaction solution from each well. Wash each well three times with 150 µl of the Diluted WB (1X Wash Buffer) each time.

8.2 Detection Antibody Binding:

1. Prepare Diluted DAb with Diluted WB 1X Wash Buffer at a ratio of 1:1000 (i.e., add 1 µl of DAb to 1000 µl of Diluted WB). Add 50 µl of the Diluted DAb to each well, then cover with Parafilm M or aluminium foil and incubate at room temperature for 60 min.
2. Remove the Diluted DAb solution from each well.
3. Wash each well four times with 150 µl of the Diluted WB each time.
   Δ Note Ensure any residual wash buffer in the wells is removed as much as possible at each wash step.

8.3 Signal Detection:

1. Add 100 µl of DS to each well and incubate at room temperature for 1 to 10 min away from light. Begin monitoring color change in the sample wells and control wells. The DS solution will turn blue in the presence of sufficient H3K4me3 product.
2. Add 100 µl of SS to each well to stop enzyme reaction when color in the positive control wells turns medium blue. The color will change to yellow after adding SS and the absorbance should be read on a microplate reader within 2 to 10 min at 450 nm with an optional reference wavelength of 655 nm.

Δ Note Most microplate readers have the capability to carry out dual wavelength analysis and will automatically subtract reference wavelength absorbance from the test wavelength absorbance. If your plate reader does not have this capability, the plate can be read twice, once at 450 nm and once at 655 nm. Then, manually subtract the 655 nm ODs from 450 nm ODs.

Δ Note If the strip-well microplate frame does not fit in the microplate reader, transfer the solution to a standard 96-well microplate.

9. Data Analysis

1. Calculate the average duplicate readings for the sample wells and blank wells.
2. Calculate % H3K4me3 change using the following formula if the samples are from treated and untreated control tests:

$$\text{H3K4me3\%} = \frac{\text{Treated (Tested) Sample OD - Blank OD}}{\text{Untreated (Control) Sample OD - Blank OD}} * 100\%$$

Example Calculation:
Average OD450 of treated sample is 0.3
Average OD450 of untreated control is 0.4
Average OD450 of blank is 0.1

$$\text{H3K4me3\%} = \frac{0.3 - 0.1}{0.4 \text{ x } 0.1} * 100\% = 66.7\%$$

For accurate calculation:

1. Generate a standard curve and plot OD value versus amount of Standard Control at each concentration point.
2. Determine the slope as OD/ng (you can use Microsoft Excel statistical functions for slope calculation), then calculate the amount of H3K4me3 using the following formulas:

$$H3K4me3\ (ng/ml) = \frac{\text{(Sample OD - Blank OD)}}{\text{Slope * sample amount (µl)}} * 1000$$

Δ Note To measure the content of H3K4me3 in total histone H3 for normalizing accuracy of the quantified H3K4me3 %, total histone H3 amount in the samples should be quantified.

10. Typical Data

Data provided **for demonstration purposes only**.

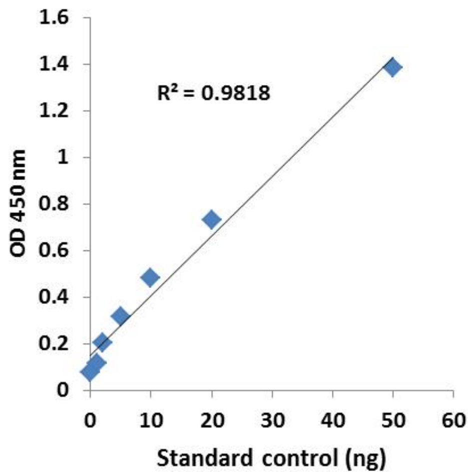


Figure 1. Illustrated standard curve.

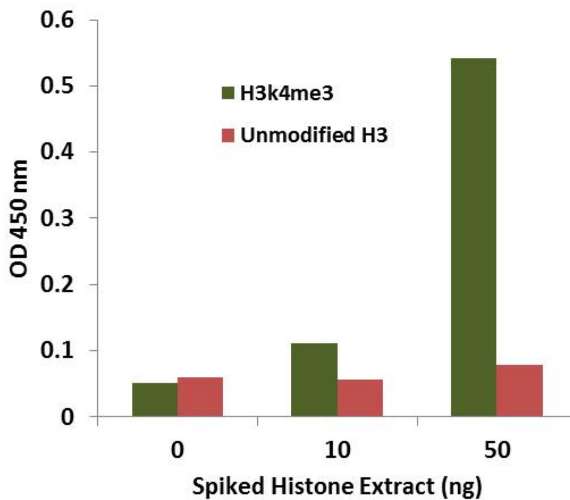


Figure 2. Histone extracts were prepared from HL-60 cells and spiked into bovine plasma at different concentrations.

11. Troubleshooting

Problem	Possible Cause	Suggestion
No signal or weak signal in both the positive control and sample wells	Reagents are added incorrectly.	Check if reagents are added in the proper order with the right amount, and if any steps in the protocol may have been omitted by mistake.
	Incubation time and temperature are incorrect.	Ensure the incubation time and temperature described in the protocol are followed correctly.
	Incorrect absorbance reading.	Check if appropriate absorbance wavelength (450 nm) is used.
	Kit was not stored or handled properly.	Ensure all components of the kit were stored at the appropriate temperature and the cap is tightly secure after each opening or use.
No signal or weak signal in only the standard curve wells	The standard amount is insufficiently added to the well in Step 8.1.3.	Ensure a sufficient amount of standard is added.
	The standard is degraded due to improper storage conditions.	Follow the Materials supplied and Storage guidance for storage of Standard Control.
High background present in the blank wells	Insufficient washing of wells.	Check if washing recommendations at each step are performed according to the protocol.
	Contaminated by sample or standard.	Ensure the well is not contaminated from adding sample or standard accidentally or from using contaminated tips.
	Incubation time with DAb is too long.	The incubation time at Step 8.2.1 should not exceed 90 minutes.

	Over development of color.	Decrease the development time in Step 8.3.1 before adding SS Stop Solution in Step 8.3.2
No signal or weak signal only in sample wells.	Sample amount added into the wells is insufficient.	Ensure a sufficient amount of plasma or serum is used as indicated in Step 8.1.4.
	Sample was not stored properly or has been stored for too long.	Ensure plasma or serum is stored in aliquots at proper temperature, for no more than 6 months.
	Little or no H3K4me3 in the sample.	This problem may be a result of many factors. If the affecting factors cannot be determined, use new or re-prepared samples.
Uneven color development	Insufficient washing of the wells.	Ensure the wells are washed according to the guidance of washing and that residue washing buffer is removed as much as possible.
	Delayed color development or delayed stopping of color development in the wells.	Ensure color development solution or stop solutions is added sequentially and consistent with the order you added the other reagents (e.g., from well A to well H or from well 1 to well 12).

12. Notes

Technical Support

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Austria
wissenschaftlicherdienst@abcam.com | 019-288-259
France
supportscientifique@abcam.com | 01.46.94.62.96
Germany
wissenschaftlicherdienst@abcam.com | 030-896-779-154
Spain
soportecientifico@abcam.com | 91-114-65-60

Switzerland
technical@abcam.com
Deutsch: 043-501-64-24 | Français: 061-500-05-30
UK, EU and ROW
technical@abcam.com | +44(0)1223-696000

Canada
ca.technical@abcam.com | 877-749-8807
US and Latin America
us.technical@abcam.com | 888-772-2226

Asia Pacific
hk.technical@abcam.com | (852) 2603-6823
China
cn.technical@abcam.com | 400 921 0189 / +86 21 2070 0500
Japan
technical@abcam.co.jp | +81-(0)3-6231-0940
Singapore
sg.technical@abcam.com | 800 188-5244

Australia
au.technical@abcam.com | +61-(0)3-8652-1450
New Zealand
nz.technical@abc.com | +64-(0)9-909-7829

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